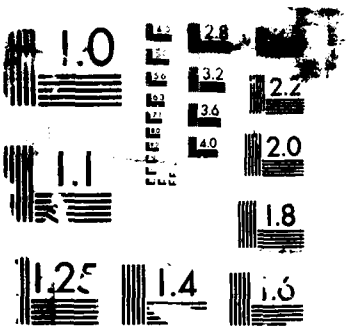
AD-A189 154 RADIOFREQUENCY/MICROWAVE CELL ABSORPTION AND ACTION SPECTROSCOPY(U) MEDICAL COLL OF VIRGINIA RICHMOND DEPT OF PHYSIOLOGY AND BIOPHYSICS S F CLEARY ET AL. 1/1

UNCLASSIFIED 10 NOV 87 N00014-84-K-0539 F/G 7/3 NL



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SECURITY CLAS

CUMENTATION PAGE

1a. REPORT SE **AD-A189 154**

1b. RESTRICTIVE MARKINGS
N/A

2a. SECURITY
N/A

3. DISTRIBUTION / AVAILABILITY OF REPORT
Distribution Unlimited

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE
N/A

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
N/A

5. MONITORING ORGANIZATION REPORT NUMBER(S)
N/A

DTIC ELECTE DEC 0 4 1987 S D

6a. NAME OF PERFORMING ORGANIZATION
Medical College of Virginia
Virginia Commonwealth Univ.

6b. OFFICE SYMBOL (If applicable)
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7a. NAME OF MONITORING ORGANIZATIO
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Arlington, VA 22217-5000

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
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8b. OFFICE SYMBOL (If applicable)
ONR

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
N00014-84-K-0539

8c. ADDRESS (City, State, and ZIP Code)
800 North Quincy Street
Arlington, VA 22217-5000

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO.	TASK NO	WORK UNIT ACCESSION NO
61153N	RR04108		

11. TITLE (Include Security Classification)
Radiofrequency/Microwave Cell Absorption and Action Spectroscopy

12. PERSONAL AUTHOR(S)
Stephen F. Cleary, Li-Ming Liu

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
~~Animal~~ Annual	FROM 9/1/86 TO 8/31/86	11/10/87	7

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06		

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
ELF electric fields, magnetic fields, tendon fibroplasia, collagen synthesis, protein synthesis, Laplace equation, member induced potential, phase transition liposomes.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Research during the contract year has included the following: a) an in vitro tendon explant model system has been used to investigate the effects of weak ELF fields on fibroplasia and collagen synthesis. Maximum (33%) fibroplasia (relative to sham-exposed explants) was induced by 1Hz square wave pulsed fields at a time averaged current density of 7mA/m^2. Under these conditions there were no effects on relative collagen synthesis. Lower or higher current densities had relatively less effects on fibroplasia. Maximal response occurred in explants oriented parallel rather than perpendicular to the E-field. At current densities of 14mA/m^2, fibroplasia and collagen synthesis were suppressed but noncollagen protein synthesis was unaffected. A series of duplicate experiments was conducted to determine the effects of pulsed magnetic fields from Helmholtz coils on tendon explant fibroplasia and collagen synthesis. No effects were detected on either dependent

(over)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)

22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
Dr. J.A. Majde	202/696-4055	ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

19. ABSTRACT (cont).

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variable. In order to relate pulsed external applied fields to cellular alterations a quasi-steady solution of Laplace's equation was obtained and programmed for computation. This solution will be used to calculate induced plasmalemma, nuclear and organelle membrane potentials and karyoplasmic and cytoplasmic potentials in response to pulsed DC electric fields and CW and pulsed RF fields. Finally, a study was conducted to determine the relative effects of 100 MHz and 2450 MHz CW RF fields on the permeability of unilamellar DDPG/DPPC liposome vesicles to cytosine arabinofuranoside in the phase-transition temperature range. Liposomes were exposed with and without fetal calf serum in the extravesicular space. RF fields at 60 W/kg had no detectable effect on liposome permeability under any exposure condition or temperature.

(Keywords :-

ANNUAL REPORT

RADIOFREQUENCY/MICROWAVE CELL ABSORPTION AND ACTION SPECTROSCOPY

Contract Number: N00014-84-K-0539

Report Period: September 1, 1986-August 31, 1987

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OVERVIEW OF RESEARCH ACCOMPLISHMENTS

During the period covered by this report the primary research accomplishments were: a) using an *in vitro* tendon explant model system a parametric study of the effects of extremely low frequency (ELF) electric fields on tendon fibroplasia and collagen synthesis was completed,

b) the tendon explant model system was used to complete a study of the effects of low intensity pulsed magnetic fields on tendon fibroplasia and collagen synthesis *in vitro*,

c) a quasi-steady state solution of Laplace's equation was obtained to permit calculation of induced electrical potentials in multilayered model of mammalian cells. Algorithms were written for digital computation of induced potentials for pulsed DC electric fields and harmonic (sinusoidal) radiofrequency electromagnetic fields,

d) a study was completed of the effects of 100 and 2450MHz CW RF radiation on the permeability of liposomes to cytosine arabinofuranoside (ARA-C) in the phase transition temperature range.

SPECIFIC OBJECTIVES

a) ELF effects on Tendon Fibroplasia and Collagen Synthesis.

Range-finding experiments were conducted to determine a ELF pulsed square wave electric field parameter set that maximally modulated tendon explant fibroplasia. This set, which consisted of 1Hz square wave modulated electric fields with a pulse duration of 1ms, was used in the majority of experiments. Sufficient data was accumulated for this parameter set to permit parametric statistical analyses.

The relationship between ELF pulsed electric field exposure was expressed in terms of the mean ratio (R) of ^{3}H-thymidine incorporation in exposed and

sham-exposed samples, normalized to tendon explant mass. Unbalanced randomized block analysis of variance of explant ^{3}H-thymidine activity revealed a statistically significant 12% increase in incorporation in samples exposed to 1-Hz, 3.5 mA/m^2 time-averaged electric field for 96h, relative to sham-exposed controls ($F(1,72) = 4.2$; $p<0.04$). A nonparametric sign test of these data yielded a p value of 0.016. The 33% average increase in ^{3}H-thymidine incorporation in samples exposed to 1-Hz, 7 mA/m^2 time averaged electric fields for 96h was highly statistically significant, as indicated by ANOVA ($F(1,32) = 37.8$; $p<0.0001$), or by a sign test ($p<0.001$). The corresponding maximum current densities and electric field strengths for these exposures were 3.5A/m^2, 2.1 V/m and 7.0 A/m^2, 4.2 V/m, respectively. Table 1 summarizes the data obtained from 5 independent experiments to determine the effect of the 1-Hz, 7 mA/m^2 time-averaged electric field parameters on tendon explant ^{3}H-thymidine incorporation.

Exposure to an average current density of 1.8 mA/m^2 resulted in a ^{3}H-thymidine incorporation ratio (R) of 0.99, which indicated no effect of electric field exposure. Explants exposed to current densities in the range of 14.0 to 57.0 mA/m^2 (the maximum time-averaged current densities investigated) exhibited a dose (current density)-dependent, decrease in proliferation relative to sham-exposed controls. ANOVA indicated that the mean decrease was not statistically significant ($F(1,45)=1.65$; $p<0.2$); however the uptake was suppressed in exposed explants in all 7 experiments. A sign test of these data indicated a statistically significant effect ($p<0.01$). Over this range of current densities pulsed electric field exposure resulted in an approximate 10% reduction in ^{3}H-thymidine uptake relative to sham exposed controls.

TABLE 1. ^{3}H-THYMIDINE INCORPORATION (DPM) IN CHICKEN TENDON EXPLANTS EXPOSED FOR FOUR DAYS TO 1Hz UNIPOLAR SQUARE WAVE PULSED ELECTRIC FIELDS: 1ms PULSE DURATION, TIME-AVERAGED CURRENT DENSITY 7 mA/m^2.

EXPERIMENT	MEAN (±SD) ^{3}H ACTIVITY (DPM)			
NUMBER	FIELD EXPOSED	# OF EXPLANTS	SHAM EXPOSED	# OF EXPLANTS
1	75,673 ± 3,329	2	34,676 ± 5,686	4
2	61,679 ± 1,833	2	58,099 ± 6,316	4
3	70,383 ± 8,212	6	67,140 ± 7,658	7
4	75,744 ± 12,205	4	58,748 ± 3,337	4
5	74,522 ± 3,260	4	68,467 ± 5,210	5

P-VALUE FOR ANOVA OF GRAND MEANS (EXPOSED VS SHAM) OVER ALL EXPERIMENTS <0.0001; SIGN TEST P-VALUE <0.001.

The effect of explant orientation, with respect to the E-field, on fibroblast proliferation was evaluated by ANOVA (unbalanced randomized block analysis) for explants exposed to average current densities of 3.5 or 7 mA/m^2. Eight experiments consisting of 4 to 6 explants per treatment group, in which the explants were oriented parallel to the E-field, yielded a $F(1,68) = 18.6$, which was statistically significant at the $p<0.0001$ level ($p<0.03$ for sign test). Parallel orientation caused an 18% average increase in ^3H-thymidine uptake. Five experiments (with the same number of explants per treatment group) with the explants aligned perpendicular to the E-field, resulted in $F(1,32) = 0.96$, which indicated no statistically significant ($p>0.3$) effect of electric field exposure on tendon explant fibroplasia.

Collagen and Noncollagen Protein Synthesis

No consistent statistically significant effects of square wave pulsed electric fields on collagen or noncollagen protein synthesis were detected at current densities of less than 10 mA/m^2, when the data were corrected for effects on fibroblast proliferation, as indicated by the relative uptake of ^3H-thymidine in exposed versus sham-exposed explants. A time-average current density of 14 mA/m^2 caused a statistically significant ($P<0.002$) 38% reduction in collagen synthesis relative to sham exposed control explants obtained from 3-4 week old chickens. Under these exposure conditions noncollagen protein synthesis was unaffected.

Relative percent collagen synthesis in sham-exposed explants was inversely proportional to donor age, varying from 31% for 3-4 week old chickens, to 14% for 8-10 week old chickens. The relatively high magnitude of these percentages is attributed to the age of the donors and the use of 1% rather than 10% FCS in the culture media during the labelling period. Explants of all ages exposed to

a 1Hz electric field at an average current density of 14 mA/m^2 had an average reduction in collagen synthesis of 41% relative to sham-exposed controls ($p<0.02$).

b) ELF Pulsed Magnetic Field Effects on Tendon Fibroplasia and Collagen Synthesis

The effects of pulsed ELF magnetic fields, generated by the EBI device with coil No. 56763, were investigated using the chicken tendon explant model system used to study the effects of square wave pulsed electrical fields described above. For each experiment five tendon explants (2mm) were placed in a 60x15mm plastic culture dish with 6ml of the same composition culture media used for pulsed electric field exposure (ie Dulbecco's Modified Eagle's Medium (DMEM) supplemented with fetal calf serum, 0.1mM ascorbic acid and 25mM Tricine buffer (pH7.4)). The coils were oriented vertically with the explant cultures positioned horizontally on the central axis of the coils. Explants were exposed in a cell culture incubator at 37 $\pm$ 0.2°C in a manner identical to and simultaneous with explants exposed to square wave pulse modulated electric fields as described in (a) above. Pulsed magnetic or electric field exposure was continued 24h/d for 3 days at which time ^{3}H-thymidine (7.4×10^5 Bq/ml) and/or ^{14}C-proline (92.5×10^3 Bq/ml) were added to the cultures and exposure was continued for 24h. The medium containing unincorporated radioisotopically labelled compounds was removed, the samples washed 3 times in Hank's Ca^{++}-free balanced salt solution and analyzed for incorporation of radiolabelled compounds. The assay procedure was identical to that used to determine the effects of pulsed electric fields on explant ^{3}H-thymidine or ^{14}C-proline incorporation. In four experiments conducted during the reporting period, pulsed magnetic fields had no detectable effect on ^{3}H-thymidine or ^{14}C-proline incorporation in tendon explants.

c) Theoretical Determination of Transmembrane Induced Potentials in Multilayered Spherical Models of Mammalian Cells.

Interpretation of cell dielectric spectroscopic data will require determination of interaction of microwave fields with cell components, principally plasmalemma, nuclear, and organelle membranes, as well as karyoplasm and cytoplasm.

A quasi-steady state solution of Laplaces equation has been obtained to provide a means of calculating these induced potentials. Algorithms for digital computer calculations have been written and implemented to calculate cell transmembrane potentials induced by exposure to square wave pulsed electric fields. Algorithms have been modified to provide induced potentials from continuous wave and pulse modulated RF and microwave fields, but these calculations have not yet been completed. After completion of this phase of the study, the equations will be modified to permit calculation of induced potentials for nonspherical (ie prolate and oblate) cell models.

d) Effects of RF Radiation on Liposome Permeability at the Phase Transition Temperature

Large unilamellar dipalmitylphosphatidylcholine (DPPC) and dipalmitylphosphitydylglycerol (DPPG) liposomes loaded with an aqueous chemotherapeutic drug, cytosine arabinofuranoside (ARA-C) were exposed for 30 min to 60 W/kg CW 100 MHz or 2450 MHz radiation in vitro over the phase transition temperature range of 37 to 43°C. Liposomes were exposed in HEPES buffer or in HEPES buffer supplemented with 44% by volume fetal calf serum (FCS). Characteristics phase transition responses (ie increased ARA-C permeability) were detected in the range of 39 to 40°C with the presence of FCS increasing maximum % release of ^{3}H-ARA-C by 20% relative to HEPES suspension. Neither frequency of electro-

magnetic radiation had any detectable effect on liposome permeability or the location of the phase transition in the presence or absence of FCS.

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7. Cleary, S.F., Liu, L.M., Graham, R. and Diegelmann R.: Modulation of Tendon Fibroplasia by Exogenous Electric Fields. Bioelectromagnetics (in press, Volume 9(2)) 1987.

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